Viral Vectors for Gene Transfer

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Following is a table listing the most common virus vectors used for gene transfer / gene therapy studies, and the biosafety levels which must be employed when producing vector stocks and/or producer lines. The table also describes the currently recommended tests for replication competent virus (RCV). In general, vector stocks and/or producer lines must be tested for RCV before being used in animal studies, and can be handled at Biosafety Level 1 (BL-1) after being tested for RCV. Finally, the table lists appropriate disinfectants for the various vector types.

Virus	Biosafety Level	Test for Replication Competent Virus	Disinfectant[10]
Oncoretrovirus (ecotropic pseudotyped)	BL-1[1]	Not required	70% ethanol, 70% isopropanol, detergent, 10% bleach
Oncoretrovirus (other pseudotypes)	BL-2	Marker rescue assay[5]	70% ethanol, 70% isopropanol, detergent, 10% bleach
Adenovirus	BL-2	Polymerase chain reaction (PCR) for E1a[6]	10% bleach
Adeno-Associated virus (with adenovirus)	BL-2	Replication-competent adenovirus[7]	10% bleach
Adeno-Associated virus (adenovirus-free)	BL-1[2]	Not required	10% bleach
Vaccinia virus	BL-2	Not applicable	10% bleach
Herpesvirus amplicons	BL-2	Plaque assay[8]	70% ethanol, 70% isopropanol,

			detergent, 10% bleach
Foamyvirus (replication competent)	BL-2	Not applicable	70% ethanol, 70% isopropanol, detergent, 10% bleach
Foamyvirus (replication defective)	BL-1[3]	Not required	70% ethanol, 70% isopropanol, detergent, 10% bleach
Lentivirus (non-HIV pseudotyped)	BL-2[4]	Serial transfer and ELISA for p24 antigen[9]	70% ethanol, 70% isopropanol, detergent, 10% bleach

(1) Oncoretrovirus vectors pseudotyped with an ecotropic envelope (one which allows for the transduction of mouse cells but not human cells) and generated by direct plasmid transfection can be handled at a BL-1 level. There is no requirement that the vector stocks and/or producer lines be tested prior to use in mice; however, a marker rescue assay[5] for RCV is recommended as part of any experimental design. Ecotropic producer cells generated by transduction with non-ecotropic pseudotyped producer cells must be handled at a BL-2 level until demonstrated to be free of RCV by a marker rescue assay [5].

(2) Adeno-associated virus vector stocks generated with adenovirus-free packaging systems can be handled at a BL-1 level, and no further testing is needed for studies in mice. Reference for adenovirus free packaging system: *Allen JM, Halbert CL, Miller AD. 2000. Improved adeno-associated virus vector production with transfection of a single helper adenovirus gene, E4orf6. Mol Ther 1:88-95.*

(3) Foamyvirus vector stocks generated with packaging systems shown to be free of RCV

by a marker rescue assay can be used in mice and handled at a BL-1 level without further testing. Foamyvirus vectors which are replication-competent must be handled at a BL-2 level. Reference for a marker rescue assay: *Trobridge GD, Russell DW. 1998. Helper-free foamy virus vectors. Hum Gene Ther 1998 9:2517-2525.* Results should be <1 infectious units/milliliter.

(4) Lentivector systems with HIV envelope gene sequences require BL-3 containment.

(5) Oncoretrovirus vectors pseudotyped with envelopes other than ecotropic must be tested for RCV by a marker rescue assay prior to use in animals and before being handled at a BL-1 level. The vector stock or producer line should be tested at a limit of sensitivity of 1 infectious unit per milliliter and the test should include a known positive control. Reference for a marker rescue assay: *Miller AD, Buttimore C. 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol Cell Biol 6:2895-2902.*

(6) The following conditions must be met before adenovirus vector stocks can be used in animals and handled at a BL-1 level. First, the vector must contain less than 2/3 of the wild-type genome; currently this only includes what are known as "gutless" vectors. Second, the vector stocks must be tested for RCV by PCR for E1a prior to use. The vector stock should be tested at a limit of sensitivity of 1 in less than 10^6 virus particles compared to a known positive control and the results of the test must be available upon request. Reference for E1a PCR assay: *Zhang WW, Kock PE, Roth JA. 1995. Detection of wild-type contamination in a recombinant adenoviral preparation by PCR. Biotechniques 18: 444-447.*

(7) Adeno-associated virus vectors generated with adenovirus must be tested for the presence of replication-competent adenovirus after heat-inactivation. Reference for a RCV assay: *Hehir KM, Armentano D, Cardoza LM, et a1. 1996. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. J Virol 70:8459-8467.*

(8) Herpesvirus generated using amplicons must be must be tested for RCV by a plaque assay prior to use in animals and before being handled at a BL-1 level. The vector stock should be tested at a limit of sensitivity of 1 infectious unit per milliliter and the test should include a known positive control. Herpesvirus vectors based on attenuated herpesvirus must always be handled at a BL-2 level. Reference for a plaque assay: *Strathdee CA, McLeod MR. 2000. A modular set of helper-dependent herpes simplex virus expression vectors. Mol Ther 5:479-485.*

(9) Lentivirus vector stocks generated with packaging systems devoid of the HIV envelope gene must be tested for RCV by serial transfer and ELISA assay for p24 antigen prior to use in animals and before being handled at a BL-1 level. The vector stock should be tested at a limit of sensitivity of 1 infectious unit per milliliter, and the test should include a known positive control. Lentivector systems with HIV envelope gene sequences require BL-3 containment. Reference for serial transfer and p24 ELISA assay: *Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. 1998. A third-generation lentivirus vector with a conditional packaging system. J Virol 72: 8463-8471.*

(10) Vectors which can be killed with 70% ethanol can also be killed by 70% isopropyl alcohol (IPA), detergents (e.g. 1% ES-7X from ICN), or 10% bleach.